

# eau d'e coli | mit igem 2006

Kate Broadbent, Andre Green,  
Stephen Payne, Veena Venkatachalam,  
Boyuan Zhu, Barry Canton, Austin Che,  
Drew Endy, Jason Kelly, Tom Knight,  
Reshma Shetty, Samantha Sutton

## overview

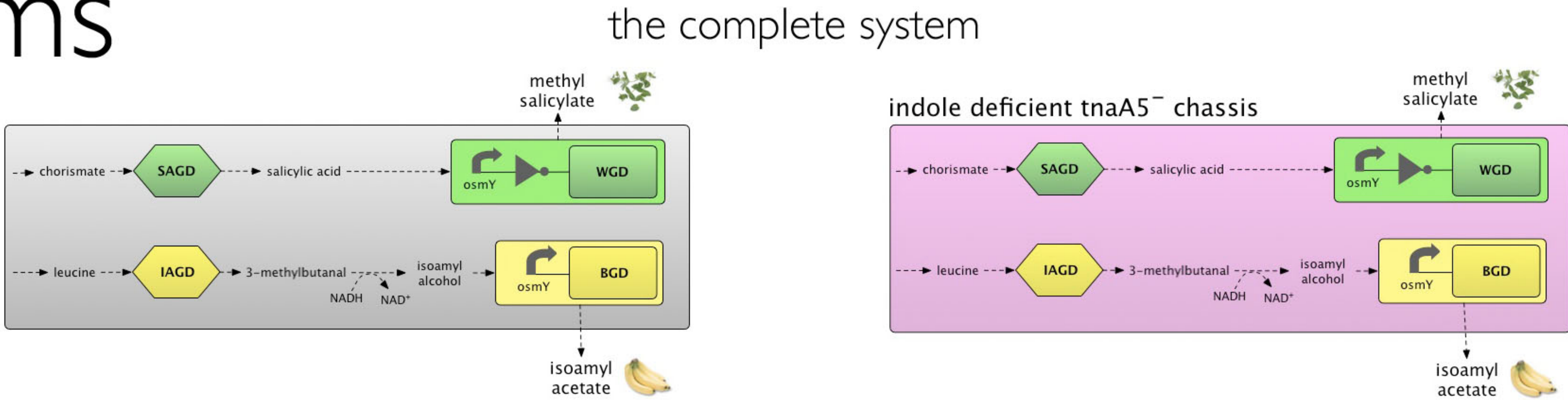
This summer, MIT's iGEM 2006 team engineered Escherichia coli to produce a wintergreen scent during exponential phase and a banana scent during stationary phase using only endogenous metabolites.

- Thus, our project demonstrates that:
1. It is indeed possible to design, build and test a synthetic biological system over the course of a summer.
  2. Biosynthetic devices that produce scented compounds can be successfully engineered in E. coli.
  3. Biosynthetic devices can be purposefully regulated via transcription based control devices.

## key contributions

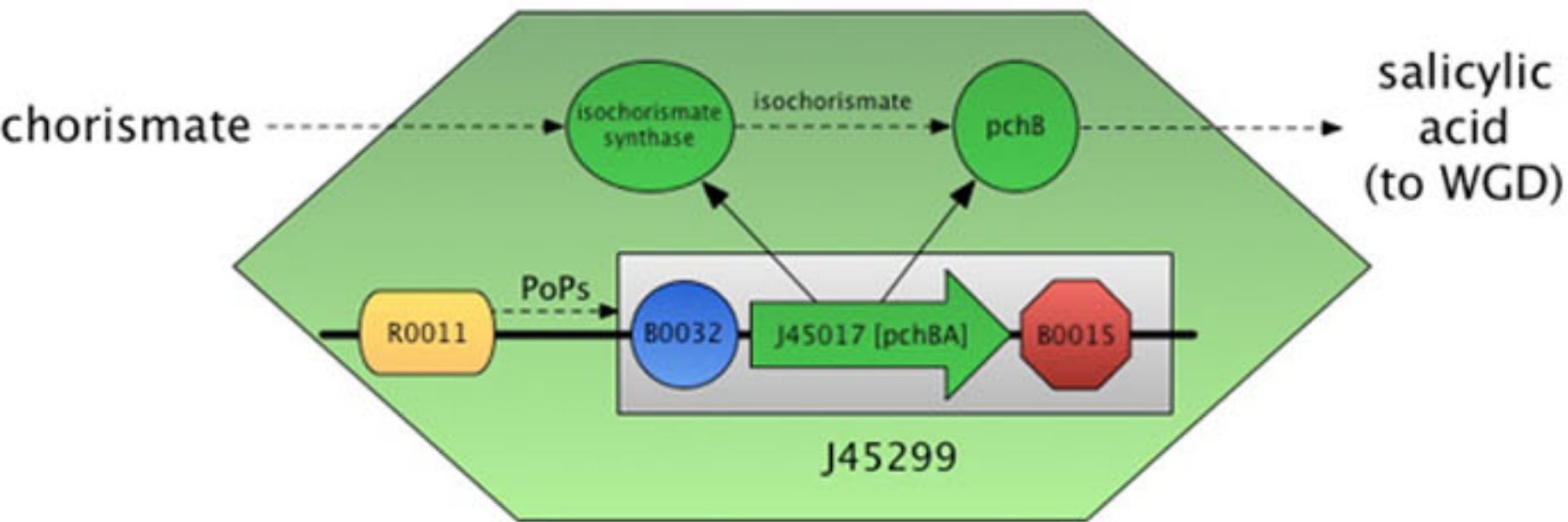
BB	Name	Description	Source	Ref.
J45017	pchBA	Salicylic Acid synthesis	P. fluorescens	(1)
J45004	BSMT	Converts S. Acid -> M. Salicylate	Petunia X hybrida	(2,3)
J45008	BAT2	1st step in Iso. Alcohol synthesis	S. cerevisiae	(4)
J45009	THI3	2nd step in Iso. Alcohol synthesis	S. cerevisiae	(4)
J45014	ATF1	Converts I. Alcohol -> I. Acetate	S. cerevisiae	(5)
J45992	osmY	Stationary Phase promoter	E. coli	(6)

## systems

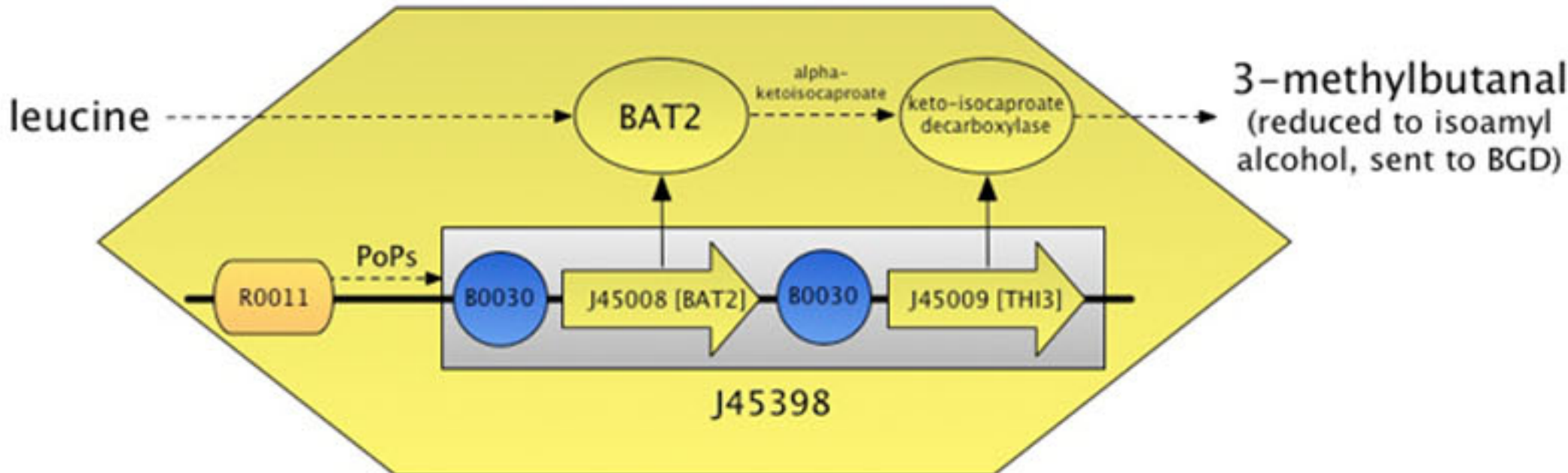


the devices

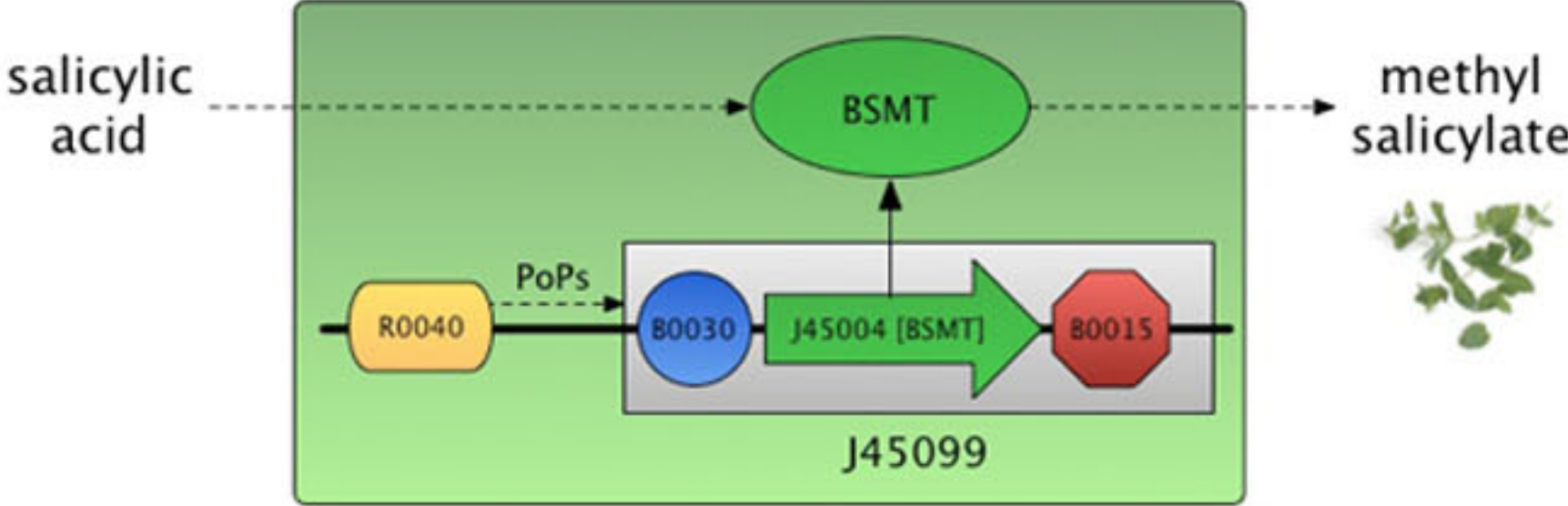
J45300 – Salicylic Acid Generating Device (SAGD):



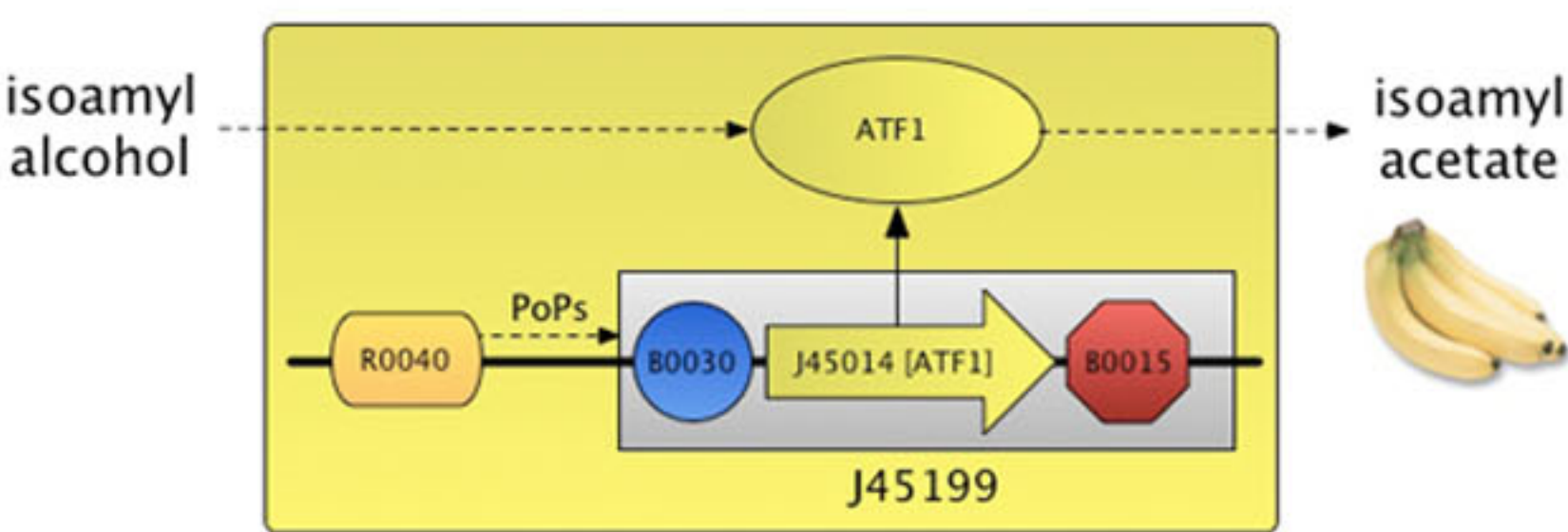
J45400 – Isoamyl Alcohol Generating Device (IAGD):



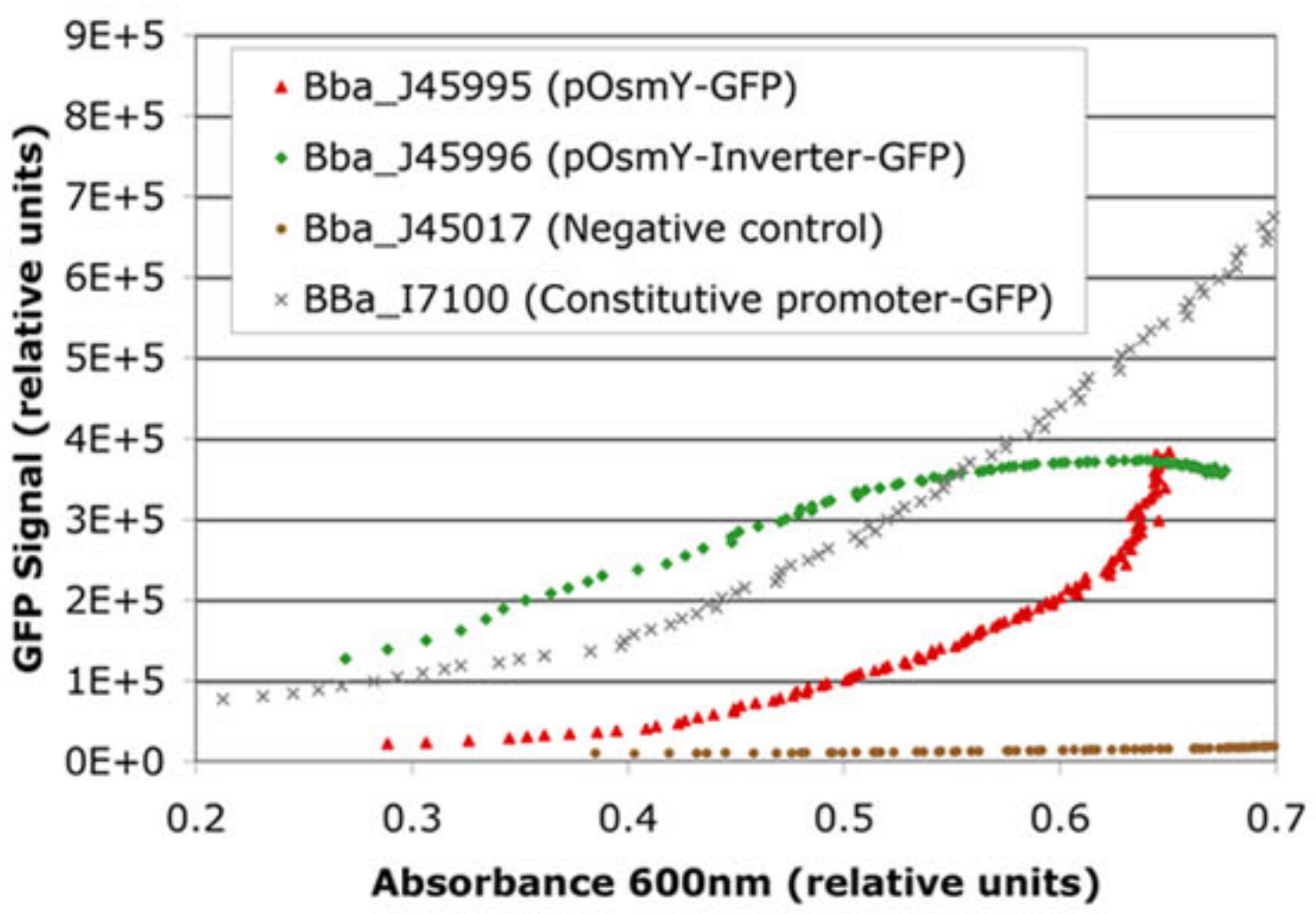
J45100 – Wintergreen Generating Device (WGD)



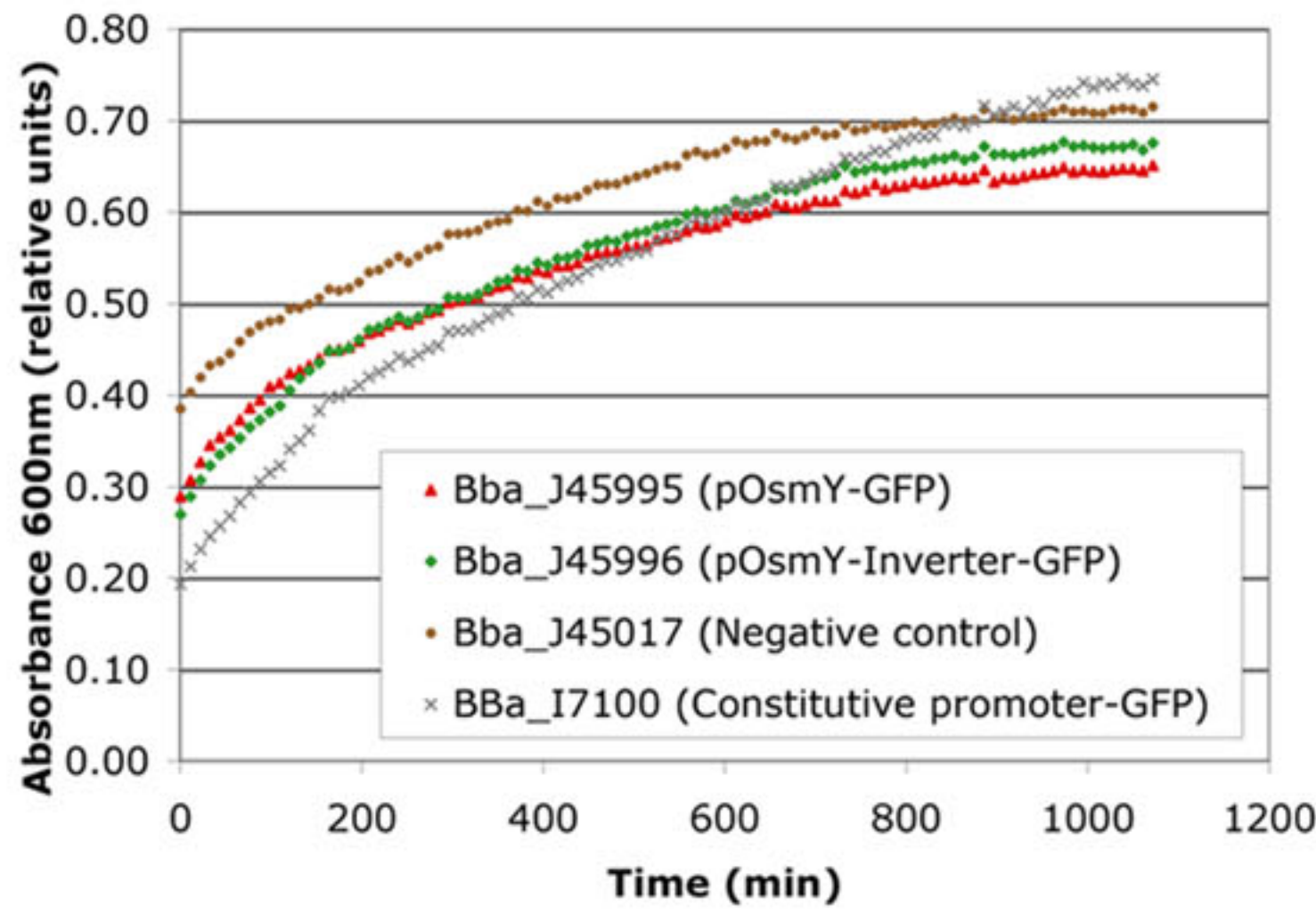
J45200 – Banana Generating Device (BGD)



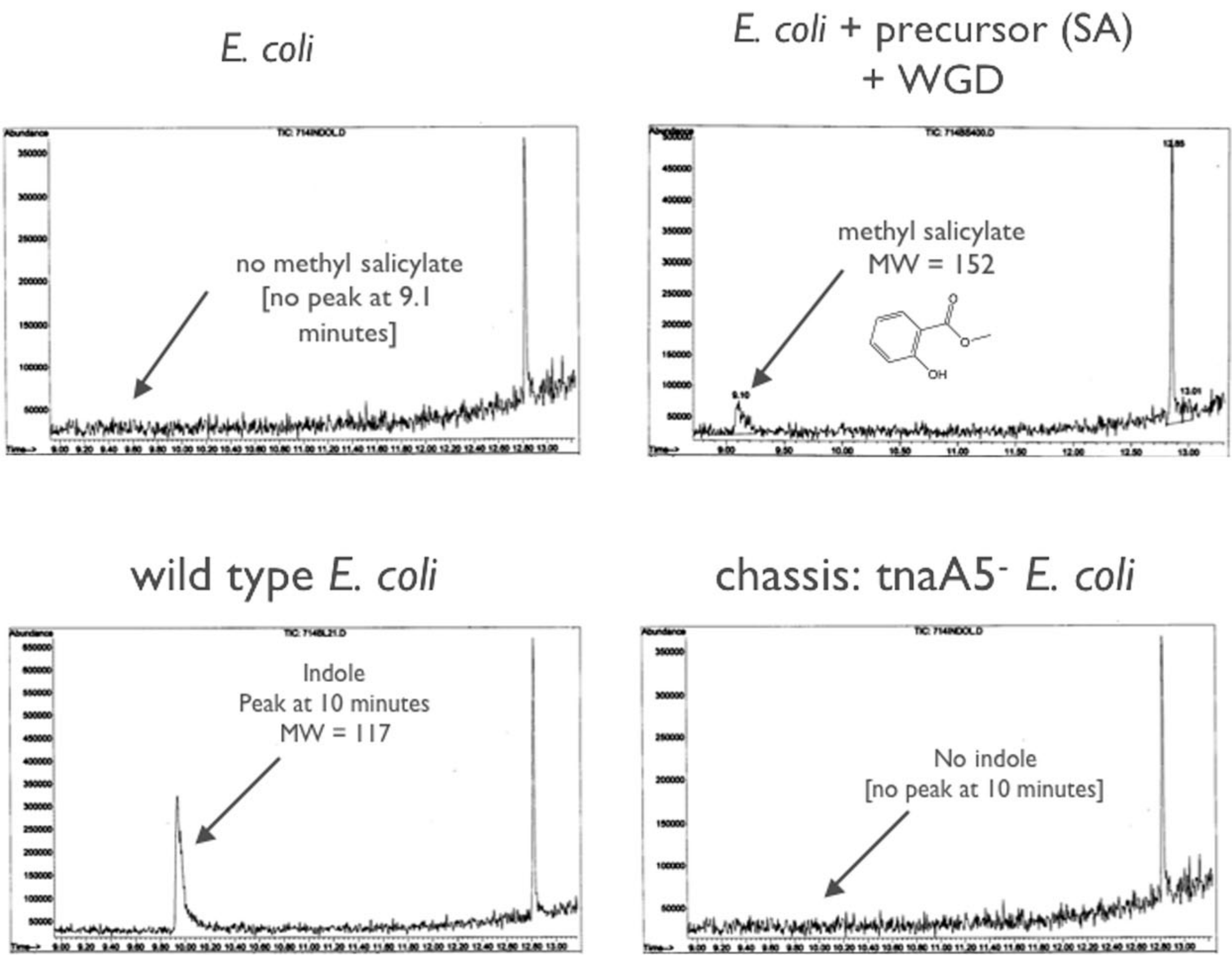
## characterization



Characterization of stationary phase regulatory mechanism using GFP fluorescence assays: J45992 [osmY] + GFP (yellow); J45992 with signal inverted by Q0401 + GFP (green); control constitutive promoter + GFP (grey); no GFP (purple).



Cell growth curves: cells reach stationary phase at absorbance ~0.5



## acknowledgments

We'd like to thank those who made invaluable contributions to this project:

Natalia Dudareva, Department of Horticulture and Landscape Architecture, Purdue University: gifts of expression vectors encoding BAMT, SAMT and BSMT enzymes.

Eran Pichersky, Department of Molecular, Cellular, and Developmental Biology, University of Michigan: suggestion of eliminating indole from Escherichia coli to mitigate the natural "bad" smell.

Mary Berlyn, Brian Cook, Cornelia Reimann, CGSC, The Coli Genetic Stock Center: Escherichia coli strain YYC912.

Dieter Haas, Department of Biological Engineering, MIT: valuable discussions.

Peter Bakker, Department of Fundamental Microbiology, University of Lausanne: gift of an expression vector carrying the pchBA coding region.

Herbert P. Schweizer, Department of Fundamental Microbiology, University of Lausanne: gift of the Pseudomonas fluorescens strain CHA0.

Pamela Silver, Utrecht University, The Netherlands: gift of an expression vector carrying the pmsCEAB coding region, as well as, gift of the Pseudomonas fluorescens strain WCS374.

Professor and Associate Head for Graduate Education and Research, Colorado State University: gift of the pUCP22 Escherichia coli to Pseudomonas shuttle vector.

Department of Systems Biology, Harvard Medical School: gift of a yeast, BioBricks vector.

## references

- (1) Mauch, F., Mauch-Mani, B., Gaille, C., Kull, B., Haas, D. and Reimann, C. (2001) Manipulation of salicylate content in Arabidopsis thaliana by the expression of an engineered bacterial salicylate synthase. Plant J. 25, 67–77.
- (2) Negre F, Kish CM, Boatright J, Underwood B, Shibuya K, Wagner C, Clark DG, and Dudareva N. Regulation of methylbenzoate emission after pollination in snapdragon and petunia flowers. Plant Cell 2003 Dec; 15(12): 2992–3006. doi:10.1105/tpc.016766 pmid:14630969
- (3) Underwood BA, Tieman DM, Shibuya K, Dexter RJ, Loucas HM, Simkin AJ, Sims CA, Schmelz EA, Klee HJ, and Clark DG. Ethylene-regulated floral volatile synthesis in petunia corollas. Plant Physiol 2005 May; 138(1): 255–66. doi:10.1104/pp.104.051144 pmid:15849311
- (4) Yoshimoto H, Fukushige T, Yonezawa T, and Sone H. Genetic and physiological analysis of branched-chain alcohols and isoamyl acetate production in Saccharomyces cerevisiae. Appl Microbiol Biotechnol 2002 Aug; 59(4-5): 501–8. doi:10.1007/s00253-002-1041-5 pmid:12172617
- (5) Horton CE, Huang KX, Bennett GN, and Rudolph FB. Heterologous expression of the Saccharomyces cerevisiae alcohol acetyltransferase genes in Clostridium acetobutylicum and Escherichia coli for the production of isoamyl acetate. J Ind Microbiol Biotechnol 2003 Jul; 30(7): 427–32. doi:10.1007/s10295-003-0070-0 pmid:12937998
- (6) Yim, H. H., R. L. Brems, and M. Villarejo. 1994. Molecular characterization of the promoter of osmY, an rpoS-dependent gene. J. Bacteriol. 176:100–107.